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# Preference and feeding specificity of *Tetrodontophora bielanensis* (Collembola, Insecta) under laboratory conditions

BY

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**Synopsis:** Food preference of *Tetrodontophora bielanensis* on 15 different fungal species have been studied in laboratory conditions. Feeding specificity was registered, pointing out the existence of different affinity of these insects toward individual fungal species.

**Keywords:** *Tetrodontophora bielanensis*, fungal diet, food preference.

## INTRODUCTION

During the last years numerous investigations were concentrated on the feeding specificities of individual Collembolan species. It has been found that these insects feed on very broad spectrum of different plant and animal materials such as: hyphae, spores and other fungal structures, bacteria, algae, fresh and decomposed plant tissues, pollen, feces and animal exuvia, etc. Results of several authors (POOLE, 1959; CHRISTIANSEN, 1964; SINGH, 1969; GILMORE and RAFFENSPERGER, 1970; ANDERSON and HEALEY, 1972; ANDERSON, 1975; CHRISTEN, 1975; McMILLAN, 1975; MARSHALL, 1978) shed some more light to the problems of Collembola feeding preference and specificity. Recent examinations of most of the authors are directed toward the studies of feeding specificity and preference of individual Collembolan species in regard to their efficiency in decomposition of organic residues of different origin.

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*Tetrodontophora bielanensis* is distributed on Fruška Gora mountain (North-Eastern Serbia, altitude above the sea level 400 m) with relatively high density of approximately 700 individuals/m². During the time period 1982-1984, productivity of this species under both natural and laboratory conditions was investigated. Examinations of the digestive tract content showed that the fungi represent the main food source for these insects throughout the whole year. The basic aim of the present study was to investigate *T. bielanensis* diet under laboratory conditions. The insects were offered different fungi as the only source of the food and this enabled us to find out the differences in the degree of consumption of individual fungal species.

## I. — MATERIAL AND METHODS

*Tetrodontophora bielanensis* (Collembola, Insecta) were grown under laboratory conditions on a natural substrate (litter fragments, mosses, lichens, tree bark, loose humus, etc.) collected from their habitats at Fruška Gora mountain. Groups consisting of 200-400 individuals were put in a glass jars (23 cm in diameter) and kept in the dark room at 10° C.

Immediately after the catching, several individuals were sacrificed and complete digestive tract together with its content was introduced into a nutrient medium with aseptic precautions. Malt, PDA or Czapek media were used throughout these studies. After 7 days at 25° C, cultures of micro-organisms originating from *T. bielanensis* digestive tract were developed. From these cultures preparations were obtained and identification of microorganisms contained in the digestive tract was performed. At the same time, using standardized microbiological methods, cultures of fungi were isolated from the forest litter collected at the habitats on the Fruška Gora and compared to those isolated from the digestive tract of these insects.

Feeding intensity of *T. bielanensis* kept under laboratory conditions was followed using 15 different species of the fungi isolated from a natural substrate (forest litter) found at the Fruška Gora. Individuals were kept without food for 24 h and after that they were divided into groups of 10 individuals which were put into glass jars (10 cm in diameter). Bottom of these jars was covered with plaster in which different fungal species were introduced. After 24 h the amount of unconsumed food and excreted feces were measured by a gravimetric method of KLEKOWSKI and DUNCAN (1975).

As the amounts of consumed food quantitatively differed depending on the fungal species, in a parallel series of experiments 17 various combinations of 3 fungal species were offered to the groups consisting of 10 individuals. Trophic activity of *T. bielanensis* was followed after 4, 6, 24 and 48 hrs by counting at each time point number of fecal particles excreted. Significance was checked by $x^2$-test.

## II. — RESULTS AND DISCUSSION

CHRISTEN (1975) established different interactions of four Collembola species with 30 different species of fungi. Examinations of the digestive tract of *Tetrodontophora bielanensis* individuals collected during different seasons at Fruška Gora mountain showed that some fungi represent the main source of food of this Collembola species. Besides conidia, hyphae, sporangiospores and other fungal structures, numerous mineral particles of different size were noticed. During some seasons 23 genera of fungi were isolated from the digestive tract of *T. bielanensis* (Tab. I).

TAB. I

**Fungi isolated from *T. bielanensis* digestive tract throughout the year**

| Fungal genera | Month | | | | | | |
|---|---|---|---|---|---|---|---|
| | I | III | IV | V | VIII | X | XI |
| Acremonium | − | + | + | − | − | − | − |
| Allescheriella | + | + | − | − | − | − | + |
| Alternaria | − | − | − | + | + | + | + |
| Aspergillus | − | − | + | + | + | + | − |
| Aureobasidium | + | + | − | − | − | + | + |
| Cerebella | + | − | − | − | − | − | + |
| Chloridium | − | − | − | − | + | + | + |
| Chrysosporium | − | − | + | + | + | − | − |
| Cladosporium | + | + | + | + | + | + | + |
| Epicoccum | + | + | − | − | − | − | − |
| Fusarium | − | + | + | − | − | + | − |
| Helminthosporium | + | − | − | − | − | + | + |
| Hirudinaria | + | + | − | − | − | − | + |
| Humicola | − | − | − | + | + | + | + |
| Lacellina | + | + | − | − | − | − | + |
| Mucor | + | + | + | + | − | − | + |
| Paecilomyces | − | − | − | + | + | − | − |
| Penicillium | + | + | + | + | + | + | + |
| Rhizopus | + | − | − | + | − | + | + |
| Saccharomyces | − | + | + | + | + | − | − |
| Scopulariopsis | − | − | + | + | − | − | − |
| Trichoderma | + | + | + | + | + | − | − |
| Xylophyla | + | + | − | − | − | − | − |

From the data listed in Table I it can be seen that genera *Cladosporium* and *Penicillium* were found it the digestive tract content almost throughout the whole year. The other fungal genera appeared periodically 2-5 times a year, depending on their seasonal appearance at the Fruška Gora habitats.

High abundance of the fungi in the digestive tract of *T. bielanensis* is directly related to micro-distribution of individuals of this insect species at the habitats on the Fruška Gora. Aggregations of individuals of *T. bielanensis* are observed the most frequently in micro-habitats which were optimal for development of certain fungi. These results are in agreement with the data of ANDERSON and HEALEY (1972) who observed quantitative differences in the abundance of individual components of the digestive tract in two species from the genus *Tomocerus (T. minor* and *T. longicornis)* as well as in *Orchesella flavescens* connected to trophic characteristics of the habitats of these species.

Parallel to analysis of the digestive tract content of *T. bielanensis* individuals from the natural populations, daily consumption of different fungal species and total fecal mass (ind/24 h) were measured in individuals kept

under defined laboratory conditions. The results of these studies are summarized in Table II.

Numerical values given in Table II show that significant differences in the amount of consumed food were registered in relation to the fungal species offered as a diet. Individuals fed on *Chrysosporium pannorum* consumed about 0.372 mg/24 h, while those fed on *Aspergillus sydowi* used about 7 times less of this fungus. The values for both number of fecal particles and total fecal mass excreted by one individual during 24 h are directly dependent on the degree of consumption of individual fungal species.

TAB. II

**Diet of *T. bielanensis* under laboratory conditions with 15 fungal species (fresh weight)**

| Fungal species | 1 | 2 | 3 |
|---|---|---|---|
| *Chrysosporium pannorum* | 0.372 | 0.030 | 9.0 |
| *Cladosporium cladosporioides* | 0.365 | 0.028 | 8.4 |
| *Penicillium variabile* | 0.331 | 0.026 | 7.7 |
| *Aureobasidium pullulans* | 0.324 | 0.025 | 7.5 |
| *Paecilomyces farinosus* | 0.312 | 0.024 | 7.3 |
| *Acremonium rulilum* | 0.285 | 0.022 | 6.7 |
| *Humicola fusco-atra* | 0.264 | 0.021 | 6.4 |
| *Mucor racemosus* | 0.248 | 0.019 | 5.8 |
| *Allernaria* sp. | 0.173 | 0.018 | 5.5 |
| *Penicillium* sp. (1) | 0.137 | 0.018 | 5.3 |
| *Fusarium oxysporum* | 0.124 | 0.017 | 5.1 |
| *Penicillium* sp. (2) | 0.105 | 0.013 | 3.8 |
| *Penicillium funiculosum* | 0.091 | 0.012 | 3.5 |
| *Trichoderma viride* | 0.060 | 0.010 | 2.9 |
| *Aspergillus sydowi* | 0.053 | 0.006 | 1.9 |

1. — mg of consumed fungi (ind/24 h).
2. — Total fecal mass in mg (ind/24 h).
3. — Number of fecal particles (ind/24 h).

Among 15 fungal species tested during our experiments, *T. bielanensis* used the most intensely *Cladosporium cladosporioides* and *Penicillium variabile* which were identified in the digestive tract content of these insects taken from natural condition almost in all seasons of the year. However, the highest daily consumption was observed when *T. bielanensis* individuals were fed on *Chrysosporium pannorum* which was recorded in the digestive tract content of the insects caught in the field during April, May and August. These results demonstrate different affinity of *T. bielanensis* toward different fungal species. However, the data concerning the correlation between the degree of consumption and chemical composition of different fungi used by *T. bielanensis* are still missing.

TAB. III

**Feeding preference *T. bielanensis* kept under laboratory conditions**

| Combinations of the fungi given | 4 h | | 6 h | | 24 h | | 48 h | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| *A. sydowi* | 0 | | 0 | | 0 | | 0 | |
| 1. *Ch. pannorum* | 17 | 34.0* | 29 | 58.0* | 106 | 200.5* | 212 | 393.8* |
| *Penicillium* sp. 1 | 0 | | 0 | | 3 | | 8 | |
| *Penicillium* sp. 2 | 0 | | 0 | | 0 | | 0 | |
| 2. *P. funiculosum* | 0 | 30.0* | 2 | 35.0* | 5 | 169.5* | 8 | 342.0* |
| *Ch. pannorum* | 15 | | 21 | | 93 | | 186 | |
| *Au. pullulans* | 7 | | 10 | | 32 | | 69 | |
| 3. *Ac. rutilum* | 3 | 4.6 | 7 | 3.8 | 18 | 14.6* | 31 | 33.4* |
| *C. cladosporioides* | 11 | | 16 | | 49 | | 97 | |
| *Pa. farinosus* | 6 | | 9 | | 43 | | 81 | |
| 4. *T. viride* | 0 | 12.0 | 0 | 19.3* | 0 | 61.8* | 0 | 115.0* |
| *Ch. pannorum* | 12 | | 19 | | 66 | | 123 | |
| *F. oxisporum* | 1 | | 3 | | 9 | | 25 | |
| 5. *A. sydowi* | 0 | 14.6* | 0 | 22.8* | 1 | 84.0* | 3 | 109.2* |
| *Pa. farinosus* | 9 | | 16 | | 58 | | 93 | |
| *Ac. rutilum* | 11 | | 17 | | 52 | | 94 | |
| 6. *Alternaria* sp. | 6 | 6.4 | 9 | 8.6 | 26 | 22.0* | 50 | 45.5* |
| *F. oxisporum* | 2 | | 4 | | 16 | | 39 | |
| *C. cladosporioides* | 6 | | 10 | | 32 | | 71 | |
| 7. *Ch. pannorum* | 10 | 3.9 | 17 | 6.8 | 56 | 19.8* | 108 | 45.1* |
| *M. racemosus* | 3 | | 5 | | 19 | | 29 | |
| *P. variabile* | 12 | | 22 | | 69 | | 128 | |
| 8. *T. viride* | 0 | 11.5 | 0 | 22.0* | 0 | 70.8* | 0 | 125.0* |
| *H. fuscoatra* | 7 | | 11 | | 32 | | 69 | |
| *C. cladosporioides* | 12 | | 17 | | 52 | | 108 | |
| 9. *P. variabile* | 9 | 6.9 | 13 | 6.4 | 41 | 15.1* | 69 | 42.8* |
| *H. fuscoatra* | 2 | | 5 | | 19 | | 31 | |
| *Pa. farinosus* | 9 | | 13 | | 38 | | 69 | |
| 10. *Au. pullulans* | 11 | 4.5 | 19 | 6.7 | 46 | 22.8* | 92 | 46.4* |
| *Penicillium* sp. 1 | 3 | | 6 | | 10 | | 19 | |
| *P. funiculosum* | 2 | | 5 | | 19 | | 43 | |
| 11. *A. sydowi* | 0 | 7.0 | 1 | 8.9 | 5 | 49.0* | 38 | 94.2* |
| *F. oxisporum* | 6 | | 11 | | 54 | | 106 | |
| *Penicillium* sp. 1 | 3 | | 6 | | 19 | | 51 | |
| 12. *Penicillium* sp. 2 | 1 | 6.5 | 2 | 7.6 | 11 | 26.5* | 33 | 31.2* |
| *P. farinosus* | 8 | | 12 | | 46 | | 92 | |
| *T. viride* | 0 | | 2 | | 6 | | 9 | |
| 13. *Ac. rutilum* | 14 | 28.0* | 26 | 44.8* | 73 | 124.7* | 137 | 241.3* |
| *A. sydowi* | 0 | | 0 | | 0 | | 0 | |

| Combinations of the fungi given | 4 h | | 6 h | | 24 h | | 48 h | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| *Alternaria* sp. | 4 | | 8 | | 21 | | 53 | |
| 14. *Penicillium* sp. 2 | 2 | 5.2 | 5 | 6.7 | 14 | 36.1* | 32 | 51.3* |
| *Au. pullulans* | 9 | | 16 | | 58 | | 111 | |
| *Pa. farinosus* | 7 | | 11 | | 36 | | 59 | |
| 15. *P. variabile* | 11 | 10.3 | 17 | 13.5 | 52 | 38.9* | 119 | 104.2* |
| *P. funiculosum* | 0 | | 1 | | 4 | | 6 | |
| *Au. pullulans* | 5 | | 11 | | 31 | | 70 | |
| 16. *C. cladosporioides* | 7 | 2.7 | 14 | 8.7 | 52 | 31.9* | 93 | 57.8* |
| *F. oxisporum* | 2 | | 2 | | 8 | | 13 | |
| *H. fuscoatra* | 5 | | 9 | | 28 | | 37 | |
| 17. *M. racemosus* | 3 | 3.3 | 5 | 6.2 | 13 | 15.2* | 23 | 51.2* |
| *Ac. rutilum* | 9 | | 16 | | 42 | | 91 | |

1. Number of fecal particles.
2. — $X^2$; Significance $^*P_{H_0} = 0.1$ %, degrees of freedom df = 2.

The results concerning food consumption when different combinations of three fungal species were given to individuals kept under laboratory conditions are listed in Table III.

Comparison of the data from Tables II and III shows that consumption intensity of individual fungal species is similar to that of their combinations. *Ch. pannorum* and *C. cladosporioides* as well as *P. variabile* alone, or in combination with other fungi represent the most attractive food of *T. bielanensis*. Consumption intensity depends on the attractivity of other fungal species given in combination and the less attractive the other two fungal species the higher degree of the consumption of the prefered fungus was registered. Trophic activity on non-attractive fungi was completely absent when attractive fungal species were given in combination with *Aspergillus sydowi, Trichoderma viride, Penicillium funiculosum* and *Penicillium* sp. (combinaisons, 1, 2, 4, 8 and 13, Tab. III). However, if favourable fungal species were omitted, then unfavourable species were consumed by *T. bielanensis*, although to a lesser degree.

Statistical analysis of the results presented in Table III showed statistically significant preference in all analyzed combinations of food offered to *T. bielanensis* in time intervals of 24 h and 48 h. In shorter feeding intervals (4 and 6 hrs) significant preference was observed only if the combinations of the fungi contained very attractive species. When the species similar by the degree of attractivity were offered, $X^2$ values obtained indicate insignificant preference. Our results could be connected to the findings of SINGH (1969) and MCMILLAN (1976) who performed similar studies related to the feeding specificity of several Collembola species and suggested direct relationship of the substrate composition and feeding specificity of individual species.

Examinations of the digestive tract content of *T. bielanensis* established

that these insects can digest hyphae and mycelia of the fungi to a certain degree. Observations of the digestive tract content and feces (light-microscopy preparations × 640) demonstrated the compactness of spore walls. After aseptical introduction of feces into a nutrient medium, cultures of the following fungi were obtained — *Cladosporium cladosporioides, Mucor racemosus, Alternaria sp., Trichoderma sp.* and *Penicillium spp.* The presence of a certain number of viable spores in the defecation products of this species needs the application of ČERVEK's (1971) and PONGE's & CHARPENTIE's (1981) very sensitive methods, in order to determine the differences in spore germination before and after passage through the digestive tract of these animals.

On the basis of our results it was found that *Tetrodontophora bielanensis* represents mycophage species with pronounced feeding specificity. It could be assumed that feeding specificity is related to organic matter composition of both fungal hyphae and mycelium, especially in regard to the chemical specificity and thickness of their cell walls.

## III. — CONCLUSIONS

*Tetrodontophora bielanensis* represents mainly a mycophage insect species. In miwed oak-hornbeam community *(Aculeato-Querco-Carpinetum serbicum B. Jov)* at Fruška Gora mountain this insect species plays in important role in decomposition of organic matters of fungal mycelium and hyphae, i.e. their return into general circulation of the matter in ecosystems.

Seasonal variations in the abundance of different fungal species in the diet of this Collembola species were observed in relation to succession of the fungi in their habitat.

Feeding specificity of *T. bielanensis* kept under laboratory conditions was registered, pointing out to the existence of different affinity of these insects toward individual fungal species, resulting in the variations of the food consumption.

The results presented in this paper suggest that preference and feeding specificity of *T. bielanensis* results from the chemical composition of organic matters in the fungal hyphae, mycelia and spore, especially from the structure and thickness of their cell walls.

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SUMMARY

Diet of *Tetrodontophora bielanensis* (Collembola, Insecta) cultivated under controlled laboratory conditions and fed on different fungal species has been studied.

Examinations of the digestive tract content of the individuals caught in the field have shown that this species feeds mainly on the fungi and that composition of the fungi in the digestive tract varies in relation to fungal succession at the habitat.

Investigations of the daily consumption of different fungal species and total fecal mass exceted (ind/24 h) showed significant differences directly related to the fungi offered to *T. bielanensis*. *Chrysosporium pannorum, Cladosporium cladosporioides* and *Penicillium variabile* alone, or in combination with other fungal species represent the most preferable food for these insects. Consumption of these fungal species was higher when they were given in combination with less attractive fungi. If *Trichoderma viride, Penicillium funiculosum, Aspergillus sydowi* and *Penicillium sp.* were offered in combination with either of favourable above mentioned species, trophic activity of *T. bielanensis* was completely absent. However, if only these less preferable species were offered, *T. bielanensis* consumed even these fungi, although to a lesser degree.


## Résumé

**Préférence et spécificité de l'alimentation de *Tetrodontophora bielanensis* (Collembola, Insecta) dans les conditions de laboratoire**

C'est dans les conditions de laboratoire que nous avons étudié l'alimentation de *Tetrodontophora bielansensis* (Collembola) élevé sur différentes espèces de champignons. L'examen du contenu du tractus digestif des individus provenant de la nature a montré que cette espèce se nourrit principalement de champignons, et que la composition de ceux-ci dans l'intestin varie selon la saison en fonction de la succession des champignons dans l'habitat.

Nous avons étudié la consommation journalière des différentes espèces de champignons (ind/24 h), ainsi que la masse totale des féces excrétées (ind/24 h). Lors de cette alimentation constituée de différentes espèces de champignons, nous avons constaté des différences significatives quant à la masse d'aliments consommés ainsi que des féces excrétées. Nous en avons conclu que *T. bielanensis* montrait une préférence pour trois espèces de champignons, *Chrysosporium pannorum, Cladosporium cladosporioides* et *Penicillium variabile* lorsque ceux-ci étaient combinés à d'autres espèces de champignons (3 espèces). Leur consommation est d'autant plus grande que les espèces mentionnées se trouvent être combinées à des espèces de champignons moins attractives. L'activité trophique de *T. bielanensis* pour *Trichoderma viride, Penicillium funiculosum, Aspergillus sydowi* et pour *Penicillium sp.* était complètement absente lorsque ceux-ci étaient en combinaison avec les espèces préférées. Cependant, si les espèces préférées sont absentes, et donc le choix diminué, *T. bielanensis* consommera ces espèces aussi, mais en moindre quantité.


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